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We continued to work on effects of oscillating electric fields on membrane functions, in particular the electric activation of Na,K-ATPase, and to develop theory of electroconformational coupling. We believe transmembrane electric fields are involved in the regulation of the internal activity of a cell and also in the cell-to-cell communications. An in depth study of Na,K-ATPase will provide useful information concerning the molecular design of a cell to sense and to transmit signals.

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1). In the a.c. activation of Na,K-ATPase, we began to obtain data for kinetic analysis and for comparing results in which ATP is the primary energy source. This comparison will examine whether the a.c. induced ion pumping activity is by a similar mechanism as the in vivo mechanism of ion pumpings. An a.c. field can also induce transfection of E. coli by plasmid DNA. This activity showed an optimal frequency at 1 Hz.

2). *In the development of the electroconformational* coupling model, we have obtained conditions in which the efficiency of the transduction of electric energy can approach that of the theoretical maximum, 100%. Other oscillating thermodynamic potentials have also been found to display similar properties. In particular, a pressure sensitive ion transporter has been made to absorb acoustic energy to pump an ion against its concentration gradient. The conversion of acoustic or mechanical signals into electric signals is an important ability of cells. This model will be called, piezo-electric coupling model and will be investigated in details.

3). Transmembrane potential induced by an oscillating electric field: The $\Delta\psi_{membr}$ generated by an a.c. field depends strongly on the frequency of the field and can be calculated using the Schwan Equation.

$$\Delta\psi_{membr} = 1.5\, a\, E_{appl} \cos\theta \,/\, [1 + (\omega\tau)^2]^{1/2} \qquad (1)$$

where $$\tau = a\, C_{membr}\, (\rho_{int} + \rho_{ext}/2) \qquad (2)$$

In the equations, $\omega = 2\pi f$, and f is the frequency of the electric field. τ, a, θ, C_{membr}, ρ_{int}, and ρ_{ext} are, respectively, membrane relaxation time, the radius of the cell (spherical), the angle between the field line and the normal to a position of interest, membrane capacitance, the resistivity of the cytoplasmic fluid, and of the external medium. We have measured the critical breakdown potential, $\Delta\psi_{crit}$, of the plasma membrane of murine myeloma cell line (Tib9) using a.c. field, by monitoring the entry of a fluorescence probe, propidium iodide, into the cells. The set up for these measurements is shown in Figure 1. This dye is weakly fluorescent in solution but becomes strongly fluorescent when it binds to DNA. Experiments were done under a

microscope by direct visual examination of single cells or by examining photographic prints. When an a.c. field reached the intensity, E_{crit}, that generated a maximal membrane potential, $\Delta\psi_{max}$, equal to or greater than the $\Delta\psi_{crit}$, the membrane was perforated at the two loci facing the electrodes. The dye diffused into the cell, giving rise to two bright, narrow, moon-like bands, which expanded to the whole cell in 1-3 minutes (Figure 2). $\Delta\psi_{crit}$'s were measured in three media of different resistivities, ρ_{ext}, (52600, 7050 and 2380 Ω cm), over the range 0.1 kHz to 300 kHz, with the field duration of 200 ms. Regression analysis based on equation (3) showed that in a medium of giving resistivity, the $\Delta\psi_{crit}$ was constant over the frequency range studied. When the capacitance of the membrane, C_{membr}, was taken to be 0.9 μF cm^{-2}, the resistivity of the cytoplasmic medium was determined to be 910-1100 Ω cm.

$$\Delta\psi_{crit} = 1.5\, a\, E_{crit} / [1 + (\omega\tau)^2]^{1/2} \qquad (3)$$

The $\Delta\psi_{crit}$ were 0.33 V, 0.48 V and 0.53 V, respectively, for the three media in decreasing resistivities. The good fit of these data to the curves calculated using the Schwan Equation indicates that the equation may be used to describe the transmembrane potential of a living cell generated by an oscillating electric field (Figure 3). Some electric parameters are summarized in Table 1.

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And several abstracts in scientific meetings, several manuscripts submitted.

Table 1 Some electric parameters of myeloma cells

Parameter	Experiment 1	Experiment 2	Experiment 3
a (μm)	6.5	6.5	6.5
ρ_{ext} (Ω cm)	52600	7050	2380
ρ_{int} (Ω cm)	n.a.	910	1100
α	0.17	0.030	0.015
τ' (μs)	13 [a]	2.5 [b]	1.3 [b]
$\Delta\psi_{crit}$ (V)	0.33	0.48	0.53
$\Delta\psi_{rms}$ (V)	0.23	0.34	0.37

Notes: n.a. means not applicable.

a: Value obtained by calculation.

b: Values obtained by optimization.

$\Delta\psi_{rms} = \Delta\psi_{crit} / \sqrt{2}$.

$\alpha = a\, G_{membr}\, \rho_{ext} /2$

$\tau = \tau' (1 + \alpha)$

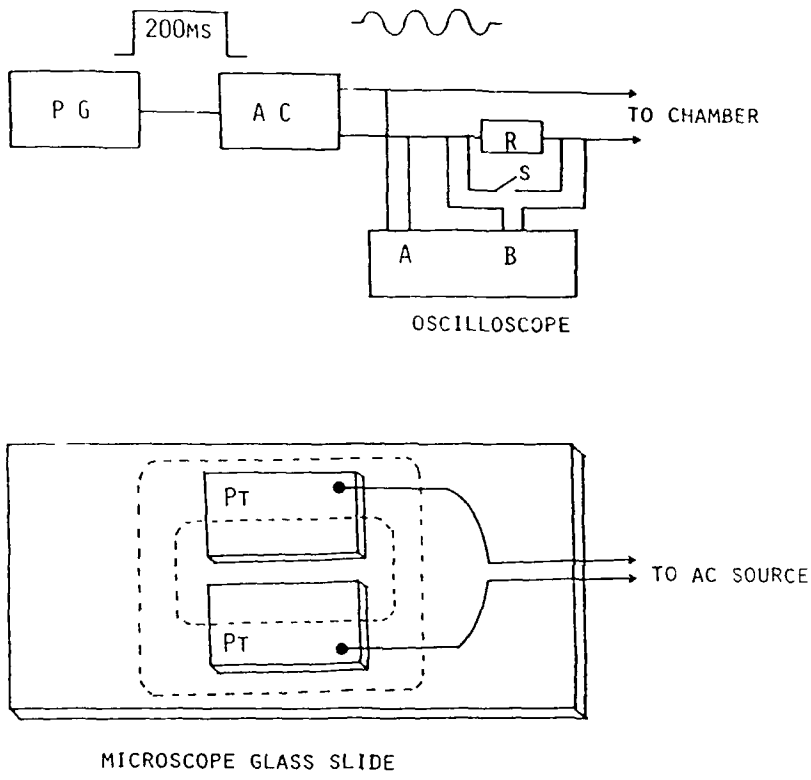

Fig. 1 Block diagrams of equipment and cell chamber for electroporation.
The upper figure is the block diagram of the equipment used for electroporation: PG denotes the pulse generator (Wavetek, Model 801); AC, the functional generator (Wavetek, Model 148A); R, a 47 Ω non-inductive resistor; and S, a switch. The lower figure illustrates the cell chamber. An a.c. field of appropriate amplitude and frequency was generated by the functional generator and applied to the cell suspension. The duration of the a.c. field, 200 ms, was controlled by a square wave triggering pulse. The distance between the two platinum electrodes was 0.1 mm. The dashed lines in the glass slide indicate the epoxy resin ring which formed a vessel to hold 150 μl of cell suspension. The conductivity of the cell suspension was monitored during the experiment by measuring the voltage drop on the non-inductive 47 Ω resistor.

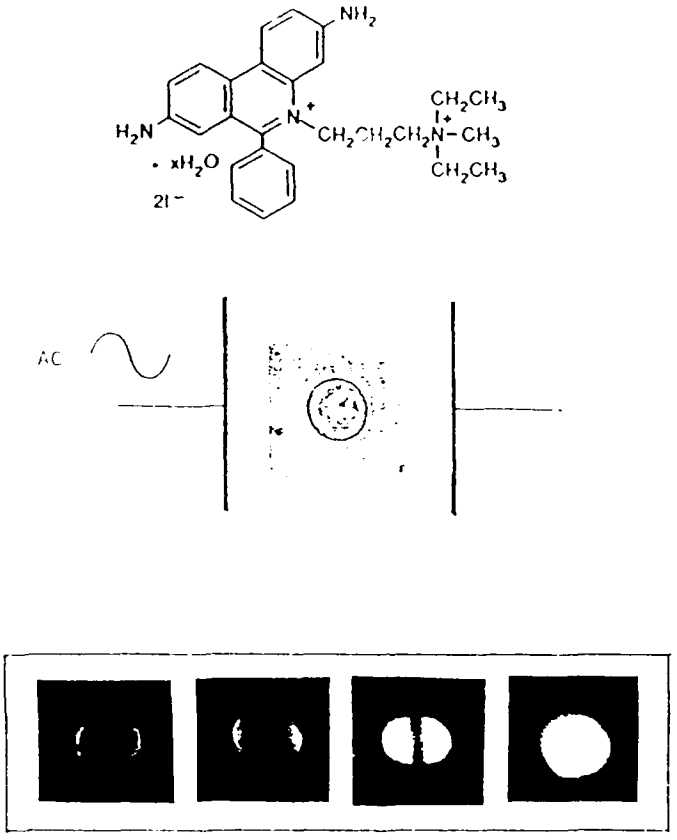

Fig. 2 Observation of electroporation by the fluorescence changes of propidium iodide

The chemical formula of propidium iodide is given in the upper figure. The middle figure shows a typical myeloma cell under the microscope (bright field). The relative positions of the platinum electrodes are indicated. The lower figure gives some photographs taken at different time after a cell was electroporated by an a.c. field, E_{crit}. Within 1-3 s, two narrow, moon-like bright bands appeared at the two loci facing the electrodes (the left most photo). The next 3 photos, from left to right, were taken at approximately 20 s, 1 min and 3 min, respectively. See text for details.

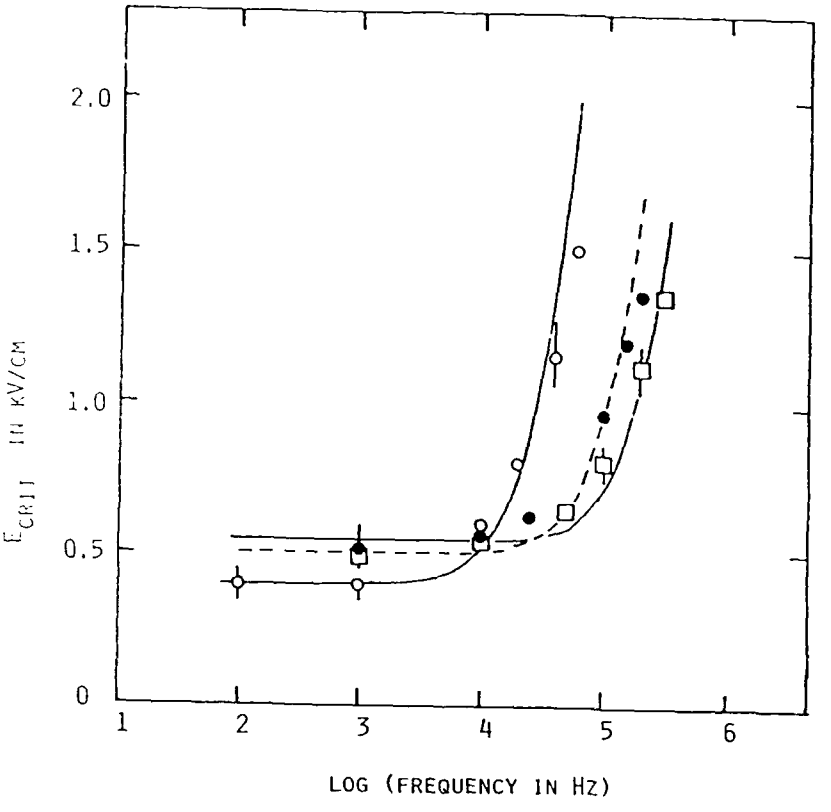

Fig. 3 Critical a.c. field strength, E_{crit}, for electroporation as a function of the a.c. frequency

Data obtained in three media of different resistivities, 52600 Ω cm (○), 7050 Ω cm (●), and 2380 Ω cm (□), are shown. The curves drawn through these data points were obtained by calculation or the optimization according to Eq. (6). See text for details.